

Microtubule-associated Proteins and In Vitro Astrocyte Differentiation

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ABSTRACT Primary cultures of mouse brain astrocytes have been used to identify the microtubule-associated proteins (MAPs) present in this cell type at different stages of in vitro differentiation. The MAPs of the astrocyte have been identified by polyacrylamide gel electrophoresis and immunological detection. Two antisera were raised against two brain MAPs, tau and MAP-2. These antisera were also used to label the microtubular network in the intact astrocytes at different stages of the culture.

The mature astrocyte contains a variety of MAP-like proteins. Anti-MAP-2 serum detected several proteins of high molecular weight (380,000, 260,000, 205,000 and 165,000 mol wt) and one microheterogeneous peak of 83,000 mol wt. Anti-tau also detected high molecular weight components (380,000 to ~200,000 mol wt) but not the 165,000-mol-wt peak; in addition two microheterogeneous peaks of 83,000 and 62,000 mol wt were detected by the anti-tau serum. The 62,000-mol-wt peak was therefore detected only by the anti-tau serum whereas the 83,000-mol-wt component cross-reacted with both antisera.

At early stages of the culture the immature cell contained about two times less immunoreactive material than at mature stages. Qualitative changes of the high molecular weight components were also observed.

In the intact cell both antisera revealed a dense fibrous network. At early stages of the culture the astroblasts were stained by the antisera but the reaction was very diffuse in the cytoplasm; few fibrous cells were intensively stained. Morphological differentiation, which began after serum deprivation and which was accelerated by forskolin (a drug that induces cyclic AMP accumulation), led to high labeling of both the cell body and the cellular processes. In the presence of colchicine the staining regressed, the processes shortened, and the cell returned to a less-apparently differentiated state.

Although less elaborated than that of the neuron, the cell form of the astrocyte changes markedly during differentiation. Astrocytes elaborate cell processes that resemble those of the neurons but they are, on the average, much shorter. Although the astrocytes develop different types of expansions and are interconnected among themselves, with the neurons, with the blood vessels, and with the basal lamina of the pial surface, their function(s) remain(s) largely unknown (for review see reference 21).

In recent years it has been increasingly clear that the shape of cells is determined to a large extent by their cytoskeleton. Neurite outgrowth, for instance, requires microtubule assembly (19, 44, 53); of particular interest have been the so-called

microtubule-associated proteins (MAPs),¹ which seem to play an important role both in neurite outgrowth and in the specification of what will be an axon or a dendrite (for review see reference 22). In contrast, very little is known about the microtubule organization of the astrocytes, their role during the differentiation process, and the growth of the cellular processes. Some data on the microtubules or the MAPs present in glioma cell lines (8, 17, 18, 39) have been published,

¹ *Abbreviations used in this paper:* A-MAP, astrocyte microtubule-associated-type protein; B-MAP-1 and B-MAP-2, brain MAP-1 and -2, respectively; B-tau, brain microtubule-associated tau protein; MAP, microtubule-associated protein.

but nothing is known of the MAPs of normal astrocytes.

MAPs co-assemble with tubulin and decorate the surface of microtubules not only in brain but also in various other tissues and cell types (see reference 22).

Brain tissue contains high molecular weight MAPs (31, 39, 48), or B-MAP-1 and B-MAP-2; and low molecular weight MAPs, or B-tau proteins (15, 16), which are themselves heterogeneous. B-MAP-2 (~300,000 mol wt), for example, has been resolved into two discrete peaks, both of which are thermostable proteins (40); at early stages of brain development B-MAP-2 is present but as a single peak (4). B-MAP-1 (~350,000 mol wt) splits into three bands (8) which are all thermolabile (27, 28) and are therefore eliminated during heat treatment of the microtubules (24). Recent developments (41) have shown that another high molecular weight MAP (MAP-4) is composed of a triplet of proteins (215,000–240,000 mol wt). B-tau, the low molecular weight MAP, is composed at an adult stage of brain development of a series of closely spaced bands of 58,000–65,000 mol wt (15, 16) which can be resolved by two-dimensional gel electrophoresis to at least 20–30 entities (40). At an early stage of brain development the low molecular weight MAPs present in the tau region clearly differ in number, molecular weight, peptide mapping, assembly promoting activity of tubulin (25, 36) and encoding mRNAs (20, 26). These “young B-MAPs” have been identified at an immature stage of development, i.e., when the brain contains few differentiated neurons and few glial cells. Little is known of the functional significance of the heterogeneity of B-MAPs and of the developmental changes that occur during brain differentiation. Some data suggest that the different MAPs are not evenly distributed within the brain and in different parts of the neurons (3, 7, 14, 29, 30, 37, 51). Some MAPs, such as MAP-2 and tau, also interact with microfilaments and neurofilaments (2, 6, 27, 34, 43, 46).

To understand better the functional significance of the heterogeneity of B-MAPs we decided to use *in vitro* culture systems of differentiating brain cells as homogeneous as possible. We report in this work the results obtained with a primary culture that has been shown to be a highly enriched population of astrocytes (1, 2, 5, 49). Such cultures begin spontaneous differentiation when deprived of serum; serum deprivation is performed when contact inhibition of growth occurs. The differentiation can be accelerated by dibutyl cyclic AMP (5) or by forskolin (this work), a drug that increases the intracellular level of cyclic AMP. Two polyclonal antibodies directed against adult B-tau and B-MAP-2, respectively, have been used (*a*) to identify the thermostable MAPs eventually present in the immature and differentiating astrocytes (A-MAPs); and (*b*) to stain the microtubule network in the intact cell at different stages of the culture.

MATERIALS AND METHODS

Products: GTP and 2-(*N*-morpholino)ethane sulfonic acid were from Boehringer Mannheim Diagnostics, Mannheim, FRG. EDTA was from Pro-labo, Paris). Forskolin was from Calbiochem-Behring Corp., La Jolla, CA. EGTA, colchicine, leupeptin, phenylmethylsulfonyl fluoride, trypsin inhibitor, benzamide, Tween-20, and bovine albumin (fraction V) were purchased from Sigma Chemical Co., St. Louis, MO. Acrylamide, bisacrylamide, SDS, and nitrocellulose sheets were from Bio-Rad Laboratories, Richmond, CA. Anti-rabbit (Ig¹²⁵I)-labeled whole antibody from donkey (specific activity, 5–20 μ Ci/ μ g) was from Amersham International, Amersham, U.K.

Cell Culture Conditions: Astroglial primary cultures were obtained as previously described (1, 9) from cerebral hemispheres of neonatal mice. These cultures contain an astroglial population constituted mostly by proto-

plasmic-like cells and by a few fibrous cells. These cells grow rapidly for 2 wk in the presence of 20% fetal calf serum and reach a spontaneous state of differentiation at around the third week of culture. Immunohistochemical staining with anti-glial fibrillary acidic protein and anti-glutamine synthetase antisera, two astroglial markers, labeled ~80% of the cell population (49). The negative labeling with neuronal markers such as glutamate decarboxylase or γ -enolase confirms the absence of any detectable neuronal contamination.

Preparation of Thermostable 100,000 g Brain Supernatants: Thermostable supernatants were prepared from adult rat brain. Brains were homogenized in 1 ml/g tissue of buffer A: 0.1 M 2-(*N*-morpholino)ethane sulfonic acid (pH 6.4) containing 0.5 mM MgCl₂, 1 mM EGTA, 0.1 mM EDTA, 1 mM GTP, 1 mM 2-mercaptoethanol, 10 mM benzamide, 5 μ M leupeptin, 25 mg/l trypsin inhibitor, and 1.5 ml/l phenylmethylsulfonyl fluoride (50 mg/ml dimethylsulfoxide). The homogenate was incubated at 4°C for 30 min and then centrifuged at 100,000 g for 60 min. The supernatant was incubated at 4°C for 60 min in the presence of 0.75 M NaCl and 2 mM dithiothreitol. The preparation was immersed in boiling water for 5 min. After centrifugation (20,000 g, 30 min), the supernatant was dialyzed overnight at 4°C against buffer A.

Protein concentration was determined as described in reference 10 with bovine serum albumin as standard.

Preparation of Astrocyte Extracts: Since most of the B-MAPs are thermostable, heat-stable extracts were prepared from the astrocytes to achieve a partial purification.

After culture medium was removed, each dish was rinsed three times with 1 ml phosphate-buffered saline (PBS). The cells were then scraped with 0.5 ml buffer A, and each lot of four dishes was rinsed with 0.5 ml of the same buffer. The suspension was homogenized at 4°C with a Potter Dounce homogenizer (model 06001, 20/55 u, Poly-Labo Block, Strasbourg, France) and treated as described above for the thermostable 100,000 g supernatant. Slab gel electrophoresis of these extracts followed by staining with Coomassie Blue showed that most of the heat-stable proteins had a molecular weight lower than ~50,000 (not shown).

Preparation of Antigens: Rat brain microtubules were purified by the temperature-dependent assembly-disassembly procedure (47, 53). B-MAPs were prepared by the method described in reference 24. B-tau was obtained by electrophoresis of the B-MAPs on SDS polyacrylamide slab gels. The same technique was applied to obtain B-MAP-2 prepared from calf brain. The bands containing B-tau or B-MAP-2 were excised from the gel, minced, and homogenized with a Potter Dounce homogenizer in the presence of 2 ml physiologic serum.

Preparation of Antisera: Antiserum to B-tau or B-MAP-2 was produced by injecting male Fauve de Bourgogne rabbits (La Clef des Champs, Olivet, France) subcutaneously with 75 μ g of corresponding antigen in complete Freund's adjuvant. The rabbits were boosted 3 and 5 wk after the first injection. Blood was collected once a week from the third week. Preimmune sera were obtained from the same animals before immunization.

Qualitative and Quantitative Gel Electrophoresis: Proteins were analyzed on a linear 4–15% polyacrylamide slab gel gradient in the presence of 0.2% SDS as described in reference 25 with some modifications. Electrophoresis was carried out overnight at a constant current of 13 mA per gel. High molecular weight proteins were analyzed electrophoretically according to the method of Laemmli (32), except that the final concentrations in the separation gel were as follows: 0.2 M Tris-HCl (pH 8.8) and 0.1% SDS.

Quantitative estimation of astrocyte microtubule-associated proteins (A-MAPs) was established at different stages of the culture by using a Vernon densitometer (Société Sidmée, Paris) equipped with a peak area integrator. Concentration series were performed to determine if the numbers obtained were within the linear response of the film.

Electrophoretic Transfer and Immunological Detection of Proteins: The procedure used for transfer of proteins from polyacrylamide gels was that of Towbin et al. (50) with several modifications. The proteins were transferred to nitrocellulose sheets at room temperature in the Bio-Rad Trans Blot Cell at a constant current of 0.25 A for 3 h. The nitrocellulose blots were cut into several vertical strips and incubated for 2 h at 50°C in 10 mM Tris-HCl (pH 7.4), 0.9% NaCl containing 3% bovine serum albumin (BSA-saline). After washing with 10 mM Tris HCl (pH 7.4), 0.9% NaCl, and 0.05% Tween-20 (saline), the blot was incubated at room temperature for 60 min with the respective antiserum diluted as required in BSA-saline. The nitrocellulose blot was then washed with agitation in saline for 90 min; the saline was changed every 15 min and at least 20 ml of saline was used for each lane. The blot was incubated at room temperature overnight with ¹²⁵I-labeled donkey anti-rabbit IgG diluted to 10⁶ cpm/ml BSA-saline containing 10% new born calf serum and 0.01% NaN₃. The blot was washed in saline as previously described and dried between two sheets of Whatman 3 MM paper (Whatman Chemical

Separation Inc., Clifton, NJ). The wet blot was then autoradiographed on Kodak X-AR5 X-ray films. After electrophoretic transfer the gels were stained with Coomassie Blue to test the efficiency of the transfer.

Cell Labeling by Indirect Immunofluorescence Procedures: Cells were extensively washed with a PBS solution and briefly rinsed with cold methanol (-20°C). Cells were then recovered with cold methanol and kept at -20°C for 5 min. Methanol was removed and the dishes were dried by air. Cells were then incubated with specific antibodies (1/50 in PBS) for 45 min at room temperature, extensively washed, and reincubated with fluorescein-conjugated F(ab')₂ fragment (Cappel Laboratories, Cochranville, PA) goat anti-rabbit (1/50 in PBS) antibodies for 45 min at room temperature. After an effective washing, plates were cut out, fixed on blades, and observed under a Leitz microscope ($\times 400$).

RESULTS

General Aspects of the Astroglial Cultures

The cells, after 7 d of culture, show a flat epitheloid shape, and some smaller well-differentiated cells have long and

branched processes. At 18 to 21 d, cells generally show advanced morphological differentiation, and the addition during that period of $1\ \mu\text{M}$ forskolin for $\sim 24\ \text{h}$ to the growth medium leads most of the cell population to look like well-differentiated astrocytes with many processes (Fig. 1, *a* and *b*). Thus, $1\ \mu\text{M}$ forskolin produces the same effects on the astroglial morphology as $0.5\ \text{mM}$ dibutyryl cyclic AMP (36).

Electrophoresis and Immunochemical Analysis of the A-MAPs

The two antisera directed against B-tau and B-MAP-2 isolated from adult brain were tested by immunoblotting towards heat-treated brain supernatants. Fig. 2 shows that the anti-B-tau serum reacted mostly with adult B-tau, with some reaction at the level of high molecular weight components. The anti-MAP-2 serum seems also to be specific for B-MAP-

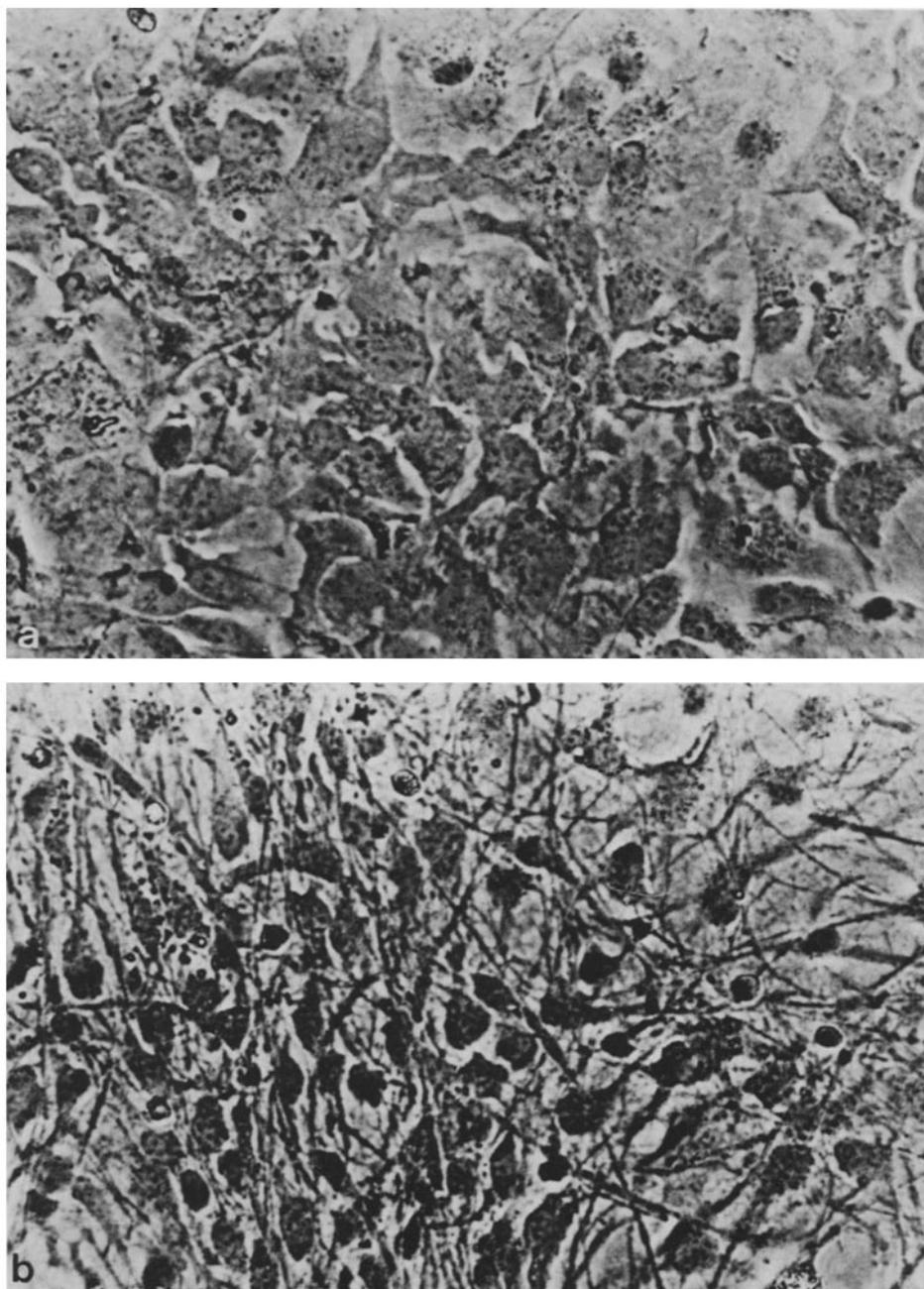


FIGURE 1 Phase-contrast micrographs of nondifferentiated (*a*) and differentiated (*b*) astrocytes. Differentiation was obtained by the addition of $1\ \mu\text{M}$ forskolin during the last 24 h of the culture, which was performed under the conditions described in Materials and Methods. $\times 200$.

2, since little cross-reactivity with B-tau was noticed (Fig. 2). In addition, some high molecular weight components other than MAP-2 reacted slightly with the anti-MAP-2 serum. Immunoblotting experiments were also performed with preimmune sera prepared from the same rabbits that were subsequently injected with the MAP-2 and tau antigens. In all cases no significant reaction was observed. These two antisera could therefore be used to identify the MAPs present

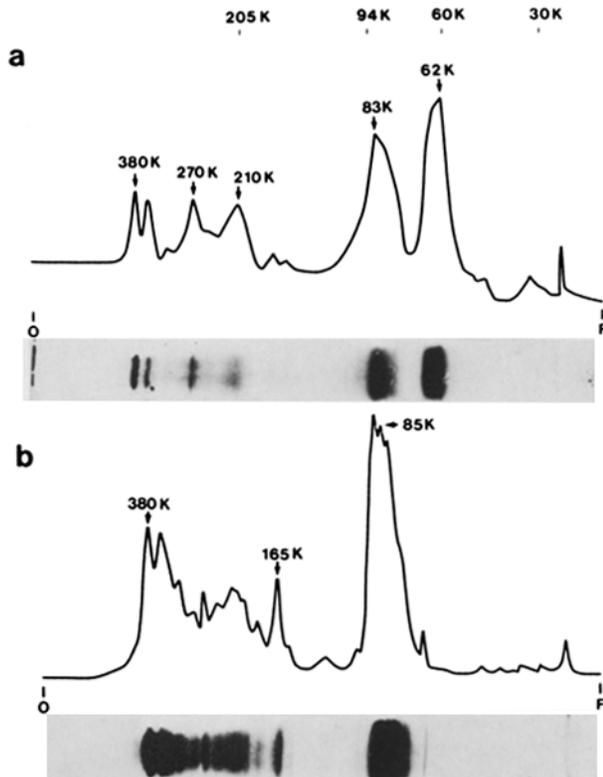
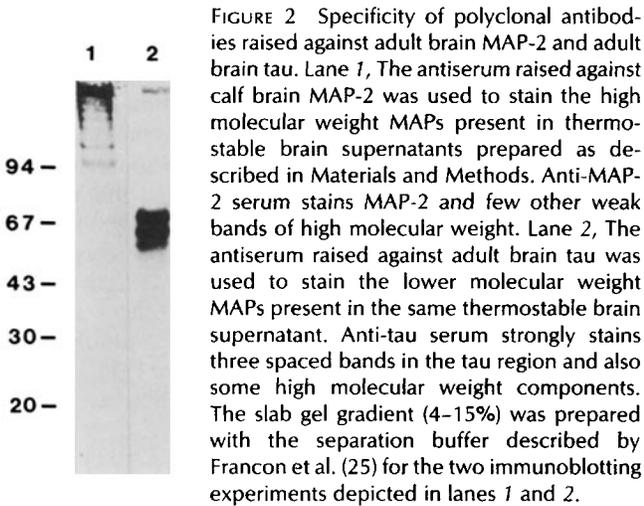


FIGURE 3 Densitometry scanning of astroglial MAP-like proteins by anti-tau (a) and anti-MAP-2 (b) sera. Also represented are the autoradiographs and the densitometer scanings of the immunoblots obtained for the thermostable extracts prepared from differentiated astrocytes. The astrocyte extract was analyzed on a 4–15% polyacrylamide slab gel gradient. The buffer used in the separation gel contained 0.2 M Tris (pH 8.8) and 0.1% SDS (the conditions that allowed a good separation of the high molecular weight components).

in *in vitro* cultured astrocytes. Thermostable extracts of morphologically differentiated astrocytes were obtained as described in Materials and Methods and analyzed by slab gel electrophoresis followed by immunoblotting. Fig. 3a shows that several astroglial protein species cross-reacted with the anti-B-tau serum. Two of them, with molecular weights of ~83,000 and ~62,000, were detected in the region where adult brain low molecular weight MAPs migrate in the same electrophoretic conditions. In contrast, Fig. 3b shows that antiserum directed against B-MAP-2 reacted only with a ~83,000-mol-wt entity. In the high molecular weight region several peaks were present whatever the antiserum used (with apparent molecular weights ranging from 380,000 to 165,000). However, the profile of the high molecular weight components in the 270,000–210,000 mol-wt region is different depending on the antiserum used; the peak at 165,000 was clearly detected by the anti-B-MAP-2 serum but not by the anti-B-tau serum. Preimmune sera prepared from the rabbits that were subsequently injected with the MAP-2 and tau antigens gave negative results.

Western blotting analyses were also performed at different stages of the culture, i.e., at various periods of the differentiation process. Fig. 4 shows that nondifferentiated astrocytes contained much less immunoreactive material than did the fully differentiated cultures. Densitometric quantitative estimations were performed from a number (three different experiments with duplicates for each experiment) of analyses similar to those depicted in Fig. 3. Fig. 5 shows that there was approximately two times less immunoreactive material in the

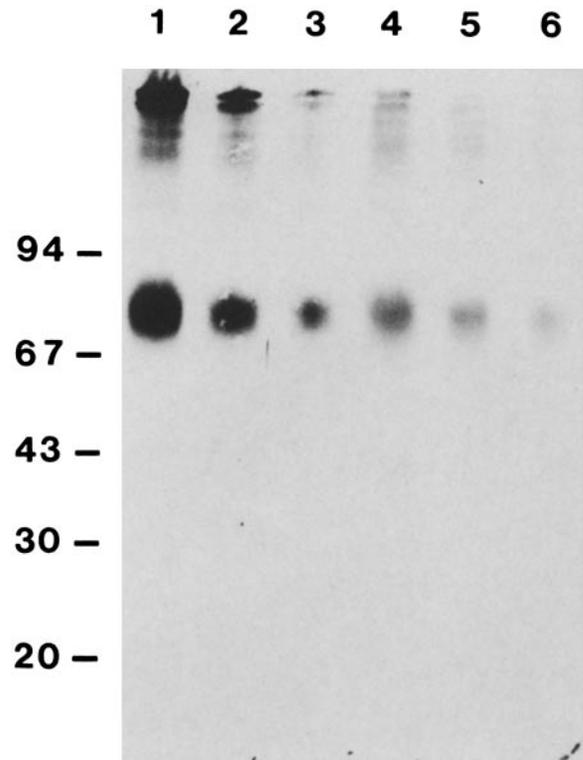


FIGURE 4 Slab gel electrophoresis of differentiated (lanes 1–3) and nondifferentiated (lanes 4–6) astroglial thermostable extracts after immunoblotting with anti-MAP-2 serum. Three aliquots of each extract were used (lanes 1 and 4, undiluted; lanes 2 and 5, diluted twice; lanes 3 and 6, diluted four times). The gel conditions were as in Fig. 2.

immature preparations than in the differentiated ones. Fig. 6 compares the densitometric profile obtained with differentiated and immature extracts containing 22 and 44 μg of protein, respectively (i.e., a double amount of protein for the immature preparation). Both with the B-tau (Fig. 6a) and with the B-MAP-2 (Fig. 6b) antisera the peaks detected at the level of the $\sim 83,000$ and $62,000$ entities are, as expected, almost perfectly superimposed; in contrast, the profiles appear to be lower at the level of the high molecular weight bands

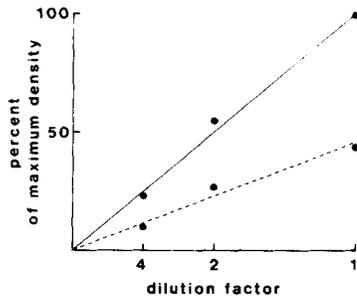


FIGURE 5 Semiquantitative evaluation of the immunoreactive material in the differentiated (—●—) and nondifferentiated (---●---) astrocytes. Slab gel electrophoresis of astroglial thermostable extract were analyzed by densitometry as described in Materials and Methods. The analysis of three aliquots (along the abscissa: undiluted (1), diluted two (2) and four times (4) (i.e. 11, 22, and 44 μg protein) of the astrocyte extract was performed on each gel. Scannings were done with two different preparations, and each preparation was analyzed in duplicate after autoradiography of the immunoblots. The results were standardized as the percentage of the maximal density (measured for the undiluted extract of the mature astrocytes). When all the numbers were calculated together and the value for the differentiated astrocytes was standardized to 100 (± 8 , $n = 12$), the value for the nondifferentiated astrocytes was 46 (± 7 , $n = 12$). This figure shows also that there is a reasonable proportionality between the different dilutions.

when the immature preparations were compared with the differentiated ones. In addition to indicating overall quantitative differences (Fig. 3), these data suggest that the concentration of the high molecular weight MAPs increases during the differentiation of the astrocytes more than that of the $62,000$ – $83,000$ -mol-wt entities.

Immunofluorescent Detection of MAP-2 and Tau-like Proteins in the Differentiating Astrocyte

Both tau and MAP-2 antigens were detectable in nondifferentiated cells (Fig. 7), whereas controls with preimmune sera were always negative whatever the period of the culture. At 7 d *in vitro*, the reaction appeared very diffuse in the cytoplasm of the flat monolayer (Fig. 7a); in contrast, few spontaneously differentiated fibrous cells, which still appear at that period, were intensely stained. After 2 wk a continuous gradient of immunofluorescence labeling intensities could be recognized, ranging from low to weakly and strongly labeled cells. Deprivation of serum at that period leads to an increase in label of some of the cells, and the addition of 1 μM forskolin as a differentiating agent for 24 h leads to a general high labeling of most of the cells (Fig. 7b). Processes as well as cell bodies show similar degrees of immunofluorescence.

The staining obtained with anti-B-MAP-2 showed approximately the same profile of evolution during the differentiation process (Fig. 8). After 2 wk of culture and in the presence of forskolin the mature cell showed a very dense network of fibers (Fig. 8b). For unknown reasons the fibrous aspect of the staining obtained with the anti-MAP-2 serum appears to be better defined than that revealed by the anti-tau serum. Anti-MAP-2 heavily stained the cell processes and the cell body. A punctuate nuclear staining seen in some cells was absent when the experiments were performed with preimmune sera.

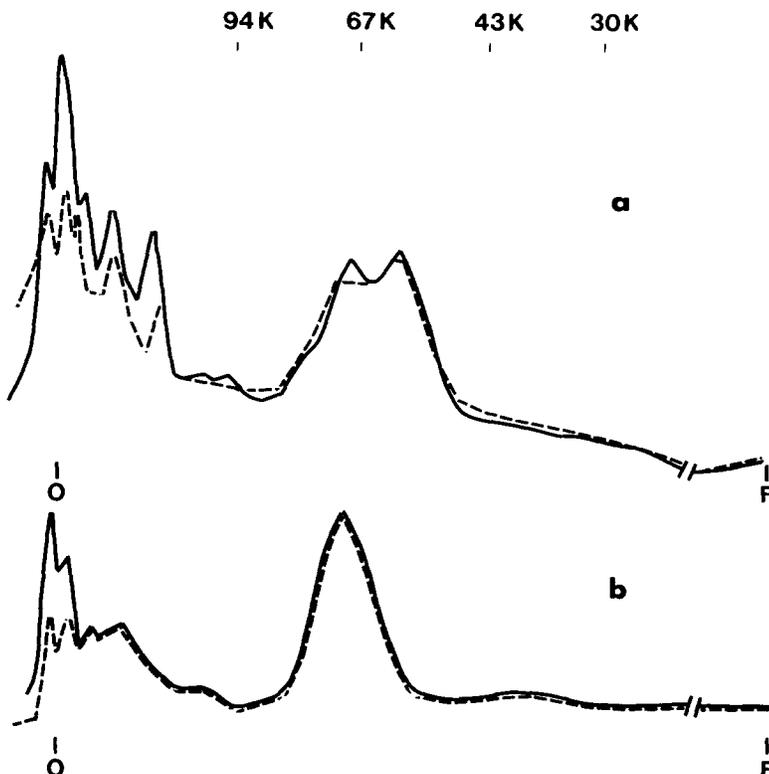


FIGURE 6 Densitometry scanning of the immunoblots obtained with anti-tau (a) and anti-MAP-2 (b) sera after PAGE (conditions of Fig. 2) of thermostable extract from differentiated (—) and immature (---) astrocytes. The extract loaded on the gel contained 22 and 44 μg proteins for the differentiated and immature preparations, respectively.

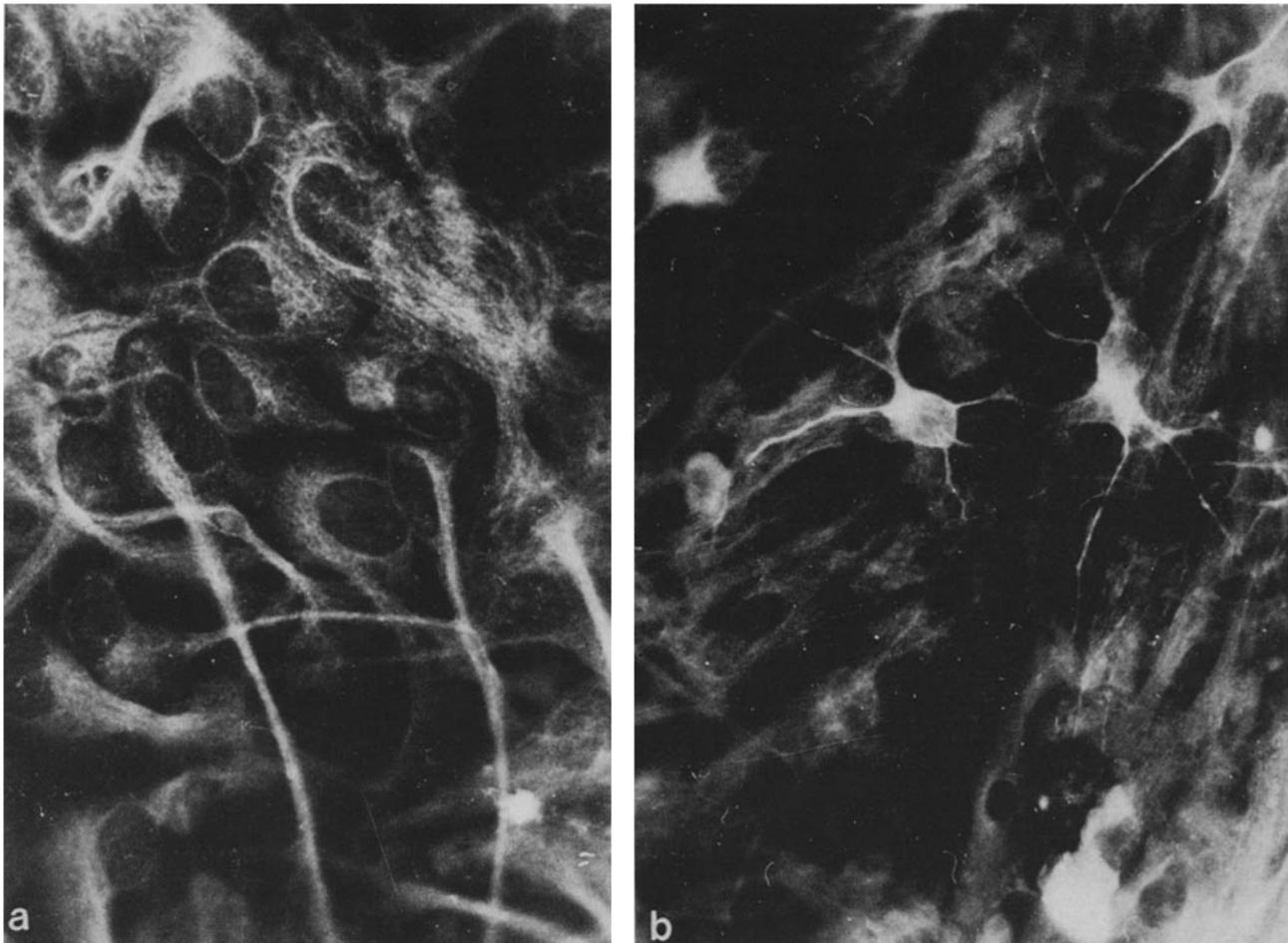


FIGURE 7 Immunofluorescence staining with anti-tau serum of cultured mouse astrocytes. (b) 7-d cells appear very weakly labeled in the flat monolayer. Few well-differentiated astrocytes appear strongly stained. (a) 18-d cells grown in the absence of serum during the last 48 h; 1 μ M forskolin was added to the growth medium during the last 24 h. Both the cell bodies and the processes appear uniformly stained \times 400.

When these cells were grown in the presence of colchicine, the staining regressed, processes shortened, and cells returned to a less apparently differentiated state (Fig. 8c).

DISCUSSION

In vitro-differentiated astrocytes contain several thermostable proteins that cross-react with two antisera raised against brain tau and brain MAP-2, respectively. In the intact cell the same antibodies react with a fibrous network which is apparently microtubular since it is disrupted in the presence of colchicine. We may therefore conclude that the astrocyte contains a variety of microtubule-associated-type proteins (A-MAPs).

A-MAPs appear to be at least as heterogeneous as the entities (B-MAPs) that co-polymerize with total brain microtubules. In addition, two groups of A-MAPs have been identified, the first with molecular weights $>200,000$, the second with molecular weights of $\sim 60,000$ – $85,000$.

To increase the probability of detection of the MAP-like entities of the astrocytes, we used polyclonal antibodies in this work. We assumed that the various MAPs might share some determinants, i.e., those related to their abilities (a) to induce tubulin assembly and to co-assemble with microtubules (for review see references 22 and 44), and (b) to interact with the calmodulin- Ca^{2+} complex (23, 33, 35). Some cross-reactivity

of both antisera towards the two antigens, B-MAP-2 and B-tau, was therefore expected. Yet the antisera used in this work appeared to be differently specific for their parent antigen, since anti-MAP-2 reacted very poorly with B-tau whereas anti-tau reacted slightly with high molecular weight entities. In contrast, each of the two antisera strongly stained both several high and low molecular weight entities present in the astrocyte extracts. However, depending on the antiserum used, the staining pattern was different in several respects: (a) Two proteins were stained by only one of the two antisera: a 165,000-mol-wt entity was detected only by anti-MAP-2, and a large microheterogeneous peak of 62,000 mol wt only by anti-tau. (b) One microheterogeneous peak of 83,000 mol wt was clearly detected by both antisera. This protein appears to be larger than any one of the different bands of adult B-tau (52,000–68,000 mol wt), which, in addition, are not significantly stained by the anti-MAP-2 serum. Thus, the 83,000-mol-wt protein appears to be specific to the astrocyte and seems to contain both the B-MAP-2 and B-tau determinants. (c) The high molecular weight components ($>200,000$ mol wt) that are stained by both antisera contain an entity of 380,000 mol wt, i.e., one much larger than any of the MAPs described so far. MAP-1, the larger MAP present in brain microtubules (40), has a molecular weight of 350,000 and is

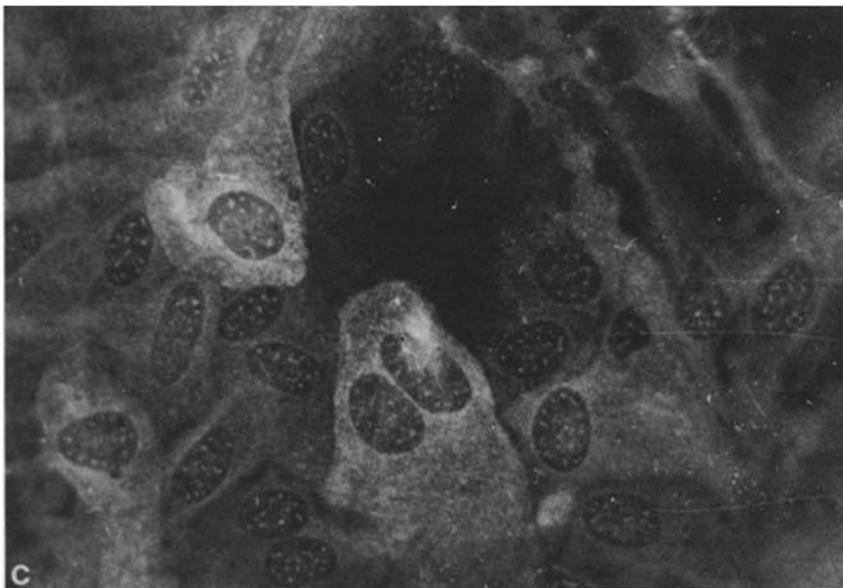
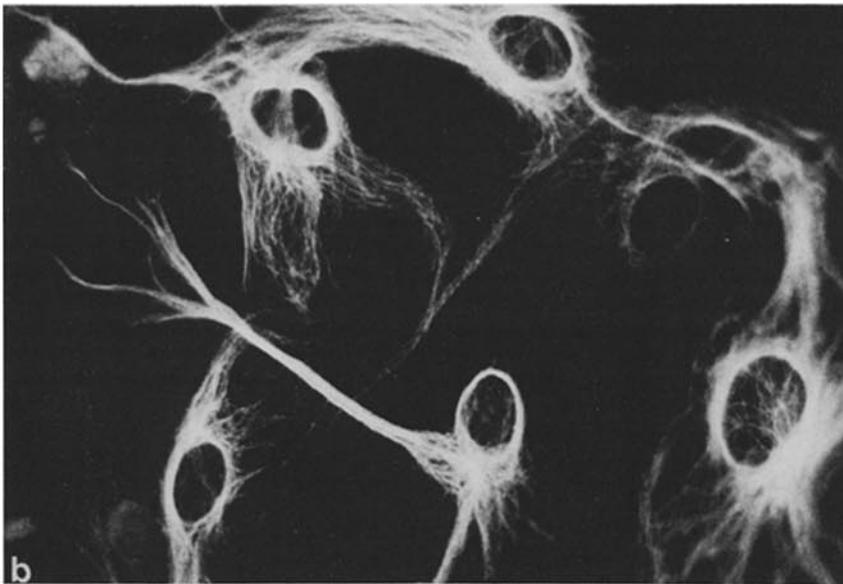


FIGURE 8 Immunofluorescence staining with rabbit anti-MAP-2 serum of cultured mouse astroglial cells. (a) 7-d cells. The flat monolayer is weakly stained. Few more differentiated cells appear heavily stained. (b) 18-d-old astroglial cells grown in the presence of 1 μM forskolin during the last 24 h; cells appear heavily labeled, showing a complex network and stained processes. (c) Same conditions as in b but in the presence of 1 μM colchicine during the last 24 h. The fibrous network disappears. Diffuse staining is restricted to the cytoplasm and the processes regress $\times 400$.

thermolabile, whereas the 380,000-mol-wt entity found in the astrocyte is thermostable. Other proteins are detected by both antisera in the high molecular weight region; the group of ~205,000–210,000 mol wt might be related to mouse MAP-4 (41, 42). An entity of the same molecular weight of 210,000, which was first isolated from HeLa cells (11, 52), has been reported to be absent from rodents (13) and to be antigenically distinct from neural MAP-2 (12). Finally, it is not even clear whether the entities that have similar molecular weights and that were detected by both antisera are the same proteins: the heterogeneous entity of 83,000 mol wt might contain, for example, different proteins, some of them sharing determinants with B-MAP-2, the others with B-tau.

The filamentous structures observed by immunohistochemical techniques in the mature astrocyte are present both in the cell pericaryon and in the cell extensions. The dividing astroblasts, which are present at earlier stages of the culture, are very poorly labeled by the antibodies. However, at these early stages few fibrous but fully differentiated astrocytes are present that contain a dense network of reactive material. At all stages such networks are sensitive to colchicine, suggesting again that the immunoreactive material they contain is formed with components associated with microtubules. This indirectly indicates that at least some of the entities identified by slab gel electrophoresis and immunoblotting are real MAPs. The staining of A-MAP-like proteins by polyclonal antibodies does not mean that the entities detected are identical to the antigens used to prepare the antisera. For instance, the presence of an ~300,000-mol-wt band, which was detected by Western blotting and by the staining obtained in the intact cell with the anti-MAP-2 serum, does not necessarily prove that the astrocyte contains a protein identical to B-MAP-2. All that can be deduced is that the astrocyte contains antigens that share some determinants with B-MAP-2. The same observations apply to the results obtained with the anti-tau serum. In this case the situation is even more clear since the immunoblotting technique did not reveal either the characteristic closely spaced four to five tau bands (15, 16) present in adult brain MAPs or the 48,000–50,000-mol-wt component seen at early stages of brain development (36). Further investigation is required to characterize fully the different protein species of the astrocyte that cross-react with the two antibodies, and to identify the thermolabile MAPs that are eventually eliminated by the heat treatment used in this work to partially purify the astroglial extracts.

Both the immunoblotting and the immunohistological techniques have been also used to follow the changes that occur during *in vitro* astrocyte differentiation. The increase in labeling seen in the intact cell seems to be well correlated with the doubling in intensity of the immunoblots. Such an increase in the amount of material that reacts with the antisera might depend at least partly on the formation of the cell processes of the astrocyte that is clearly seen during the culture. However, the cell body of the differentiated astrocytes also appears to be stained more heavily than that of the immature astroblasts. On the other hand the basal values obtained by the immunoblotting analysis at early stages of the culture should include both the weak reactivity seen at the level of the astroblasts and that of the strongly reacting material present at the level of the few differentiated astrocytes. Thus, the doubling in immunoreactivity seen during the differentiation process should be considered as a minimal figure.

In conclusion, what seems clearly established is that the astrocyte contains a heterogeneous population of proteins that cross-react with the antisera raised against the two main B-MAPs. Several of these astroglial entities differ in several respects from the total B-MAPs (molecular weight, immunoreactivity towards the two antibodies). Marked quantitative changes also occur during the differentiation process of the astrocytes; such changes seem to be required to build up a denser microtubule network in the differentiated cells than in the dividing immature astroblasts. The regression of the cellular processes seen in the presence of colchicine also suggests that, as for the neurons (19, 45, 54), microtubule assembly might be a parameter of utmost importance for astroglial differentiation.

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